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Original Article

T-cell lymphoma complicated by myelofibrosis: report of three cases and review of literature

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Abstract: Primary myelofibrosis (MF) is a rare cancerous condition that disrupts normal blood cell production and causes scar tissue to accumulate within the bone marrow. MF complicating malignant lymphoma is rare and its cause often remains unknown, but, like primary MF, its pathogenesis is likely related to cytokines produced by proliferating megakaryocytes, monocytes, and histiocytes. We have experienced three cases of peripheral T-cell lymphoma (PTCL) complicated by MF. In each case, no evidence of central nervous system infiltration was found on admission; however, bone marrow infiltration was present. We carried out a chronological analysis of the cytokine production by malignant cells in these three patients by means of lymph node and bone marrow immunostaining. Malignant cells from lymph nodes and bone marrow sampled before treatment stained positive for cytokines: platelet-derived growth factor (PDGF) and tumor necrosis factor-α (TNF-α) in case 1; PDGF in case 2; and PDGF, TNF-α, and transforming growth factor-β in case 3. These cytokines were considered to have been the cause of MF in each case. A literature search indicated that 16 cases of T-cell lymphoma complicated by MF (including our three cases) have been reported to date. Data on cytokine production by malignant cells given in these reports are summarized. Much remains to be clarified concerning the role of cytokines in the development of MF. Consequently, we plan to carry out chronological examinations of cytokines in the serum and immunostaining for cytokines in cases of PTCL and malignant lymphoma, both those complicated and not complicated by MF.

Keywords: T-cell lymphoma, bone marrow fibrosis, cytokine

Introduction

Primary myelofibrosis (MF) is a rare cancerous condition that disrupts normal blood cell production and causes the slow build up of scar tissue within the bone marrow. The pathogenesis of primary MF is likely related the production of cytokines such as transforming growth factor-β (TGF-β), basic fibroblast growth factor (b-FGF), vascular endothelial growth factor (VEGF), and tumor necrosis factor-α (TNF-α) by proliferating megakaryocytes, monocytes, and histiocytes [1-3]. MF complicating malignant lymphoma is rare and its cause remains unknown in many cases. However, several previously published articles [4-12] and some of our previous studies suggest that MF in patients with malignant lymphoma is also attributable to cytokines such as TGF-β [13-16], b-FGF [2, 17], platelet-derived growth factor (PDGF) [2, 14-16], and/or TNF-α [16], and that its pathogenesis is similar to that of primary MF.

At Juntendo University Urayasu Hospital, Japan, we have encountered three cases of peripheral T-cell lymphoma (PTCL) complicated by MF (including two cases reported by us in 2013 and 2015 [2, 16]). To help clarify the cause of MF for each case, we carried out a chronological analysis of the cytokine production by malignant cells by means of lymph node and bone marrow immunostaining. Immunohistochemistry yielded positive staining for PDGF and TNF-α in case 1; for PDGF alone in case 2; and

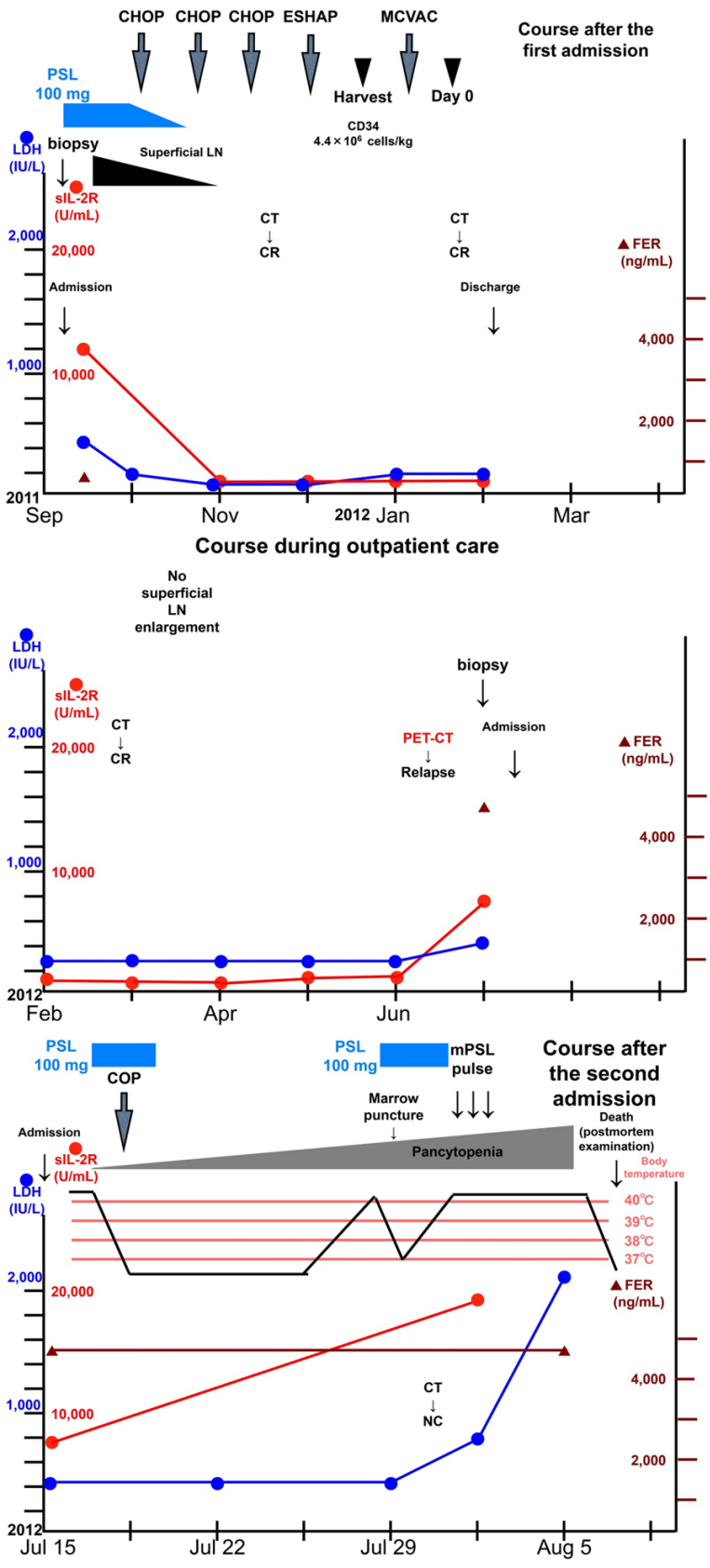


Figure 1. Clinical course of case 1. PSL, prednisolone; CHOP, cyclophosphamide, adriamycin, vincristine, prednisolone; ESHAP, etoposide, cisplatin, cytarabine, methylprednisolone; MCVAC, ranimustine, cytarabine, etoposide, cyclophosphamide; LDH, lactate dehydrogenase; sIL-2R, soluble interleukin-2 receptor; LN, lymph node; CT, computed tomography; CR, complete remission; FER, ferritin; PET-CT, F18-fluorodeoxyglucosepositron emission tomography/computed tomography; COP, cyclophosphamide, vincristine, prednisolone; mPSL, methylprednisolone; NC, no change.

for PDGF, TNF-α, and TGF-β in case 3. Marrow infiltration was noted in all three cases. Therefore, in all three of our cases, MF seems to have been induced by PDGF, TNF-α, and/or TGF-β produced by lymphoma cells infiltrating the bone marrow. The investigations and treatments carried out in our three cases will now be described.

Case presentation

Case 1: This case is newly described here. A 64-year-old man presented to Juntendo University Urayasu Hospital with the chief complaint of fever. His medical history included a gastric ulcer and external hemorrhoids. The family history was non-contributory. The patient attended our department with fever, multiple superficial lymph no-de enlargements, bilateral pleural effusion, ascites, and splenomegaly in September 2011 (**Figure 1**). Test data in **Table 1**. A cervical lymph node biopsy was performed; histopathological examination of sections stained with hematoxylin and eosin (HE) revealed loss of the basic architecture of the lymph nodes and proliferation of medium- to large-sized atypical polymorphic cells either in a cord-like arrangement or in an isolated scattered distribution (**Figure 2A**, **2B**). Immunohistochemical examination revealed pos-

Table 1. Test data for Case 1

Blood count			
WBC	9700/μL↑	Aty Ly	2.5%↑
Neut	76.5%↑	Hb	12.7 g/dL↓
Lym	13.5%↓	MCV	87.3 fl
Mono	5.0%↑	MCH	30.6 pg
Eos	2.0%	Plt	301,000/μL
Bas	0.5%		
Aggregation			
PT activity	84%	FDP	11.3 μg/mL↑
PT-INR	1.10	DD	4.03 μg/mL↑
APTT	31.4 s	AT	3.65%↓
Fbg	222 mg/dL		
Biochemistry			
TP	7.9 g/dL	AST	25 IU/L
ALB	2.6 g/dL↓	ALT	14 IU/L
BUN	24 mg/dL↑	LDH	422 IU/L↑
Cr	1.23 mg/dL↑	ALP	327 IU/L
Na	135 mM/L↓	γ-GTP	19 IU/L
Cl	100 mM/L	Ch-E	121 IU/L↓
K	4.3 mM/L	AMY	33 IU/L↓
Ca (corrected)	10.4 g/dL↑	CRP	1.2 mg/dL↑
Glu	96 mg/dL	FER	361.8 ng/mL
T-Bil	0.5 mg/dL		
Immunoserology			
IgG	3671 mg/dL↑	HTLV-1Ab	(-)
IgA	319 mg/dL	HIV1/2Ab	(-)
IgM	934 mg/dL↑	Soluble IL-2 receptor	11,600 IU/L↑

↑, upper limit; ↓, lower limit. WBC, white blood cells; Neut, neutrophil; Lym, lymphocyte; Mono, monocyte; Eos, eosinocyte; Bas, basophil; Aty Ly, Atypical lymphocyte; Hb, hemoglobin; MCV, mean corpuscular cell volume; MCH, mean corpuscular cell hemoglobin; Plt, platelets; PT, prothrombin time; PT-INR, prothrombin International Normalized Ratio; APTT, activated partial thromboplastin time; Fbg, fibrinogen; FDP, fibrin/fibrinogen degradation products; DD, D-dimer; AT, antithrombin; TP, total protein; ALB, albumin; BUN, blood urea nitrogen; Cr, creatinine; Glu, glucose; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; γ GTP, P-guanosine triphosphate; Ch-E, cholinesterase; AMY, amylase; CRP, C-reactive protein; FER, ferritin; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; HTLV, human T-cell leukemia virus; HIV, human immunodeficiency virus; sIL-2R, soluble interleukin-2 receptor.

itive staining of the tumor cells for cluster of differentiation (CD) 3, CD4, programmed cell death (PD) 1, and chemokine (C-X-C motif) ligand 13 (CXCL13) (**Figure 2C-F**), and the chromatic response of CD21 suggested marked hyperplasia of the follicular dendritic cells around the capillaries (**Figure 2G**). Staining for CD10 was negative (**Figure 2H**). Positive staining for Ki-67, a cellular marker for proliferation, was noted in a large percentage of cells (**Figure 2I**). On the basis of these findings, the patient was diagnosed as having angioimmunoblastic lymphoma (AITL).

Cerebrospinal fluid examination revealed no signs of central nervous system infiltration. Examination of a bone marrow sample obtained by aspiration revealed infiltration of the marrow by AITL (**Figure 3B13**, **3B14**), and silver impregnation staining revealed MF (**Figure 3B15**, **3B16**). The marrow blood tested negative for the Janus kinase 2 (JAK-2) V617F mutation (data not shown). The clinical stage of the disease was judged as IVB, and the Prognostic Index for PTCL-unspecified (PIT) [18] was group 4. The patient was started on cyclophosphamide, adriamycin, vincristine, and prednisolone (CHOP) therapy in early October 2011. Complete remission was confirmed by a computed tomography (CT) scan carried out at the end of the third course of CHOP therapy in mid-November 2011. Marrow testing was not performed at this time because the patient refused consent for the procedure. Up front autologous peripheral blood stem cell transplantation (auto-PBSCT) was performed. Complete remission was confirmed again by CT in February 2012. Positron emission tomography/computed tomography was performed in June 2012. This investigation revealed bilateral lymph node enlargement in the neck, axillae, and inguinal regions; enlarged lymph nodes in the mediastinum, abdomen, and pelvis; and splenomegaly. In July 2012, a right inguinal lymph node biopsy was performed, and the results confirmed relapse of the disease. After CHOP therapy, the patient's condition was complicated by hemophagocytic syndrome. Steroid pulse therapy was instituted; however, the patient showed no improvement and died of his disease in August 2012. The bone marrow was free of AITL infiltration (**Figure 3C27**, **3C28**) and MF (**Figure 3C29**, **3C30**) at the time of relapse.

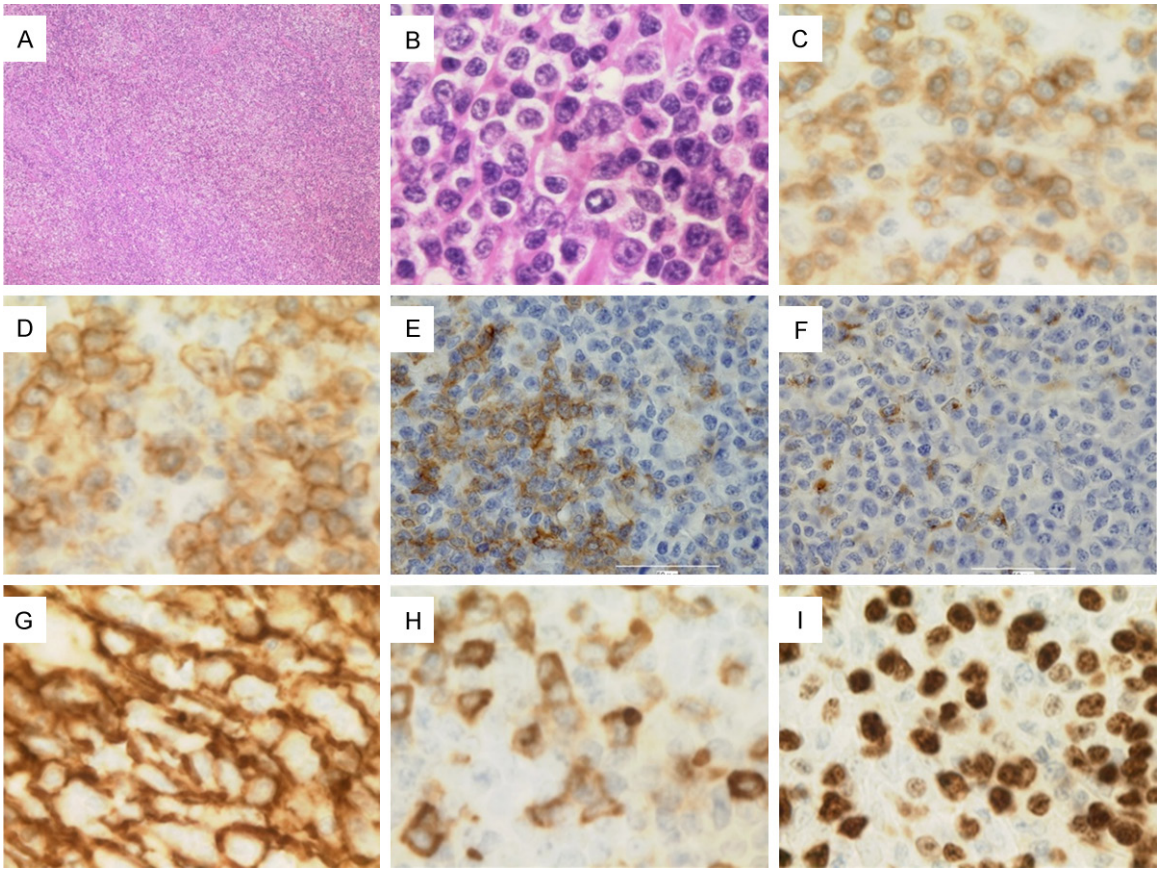
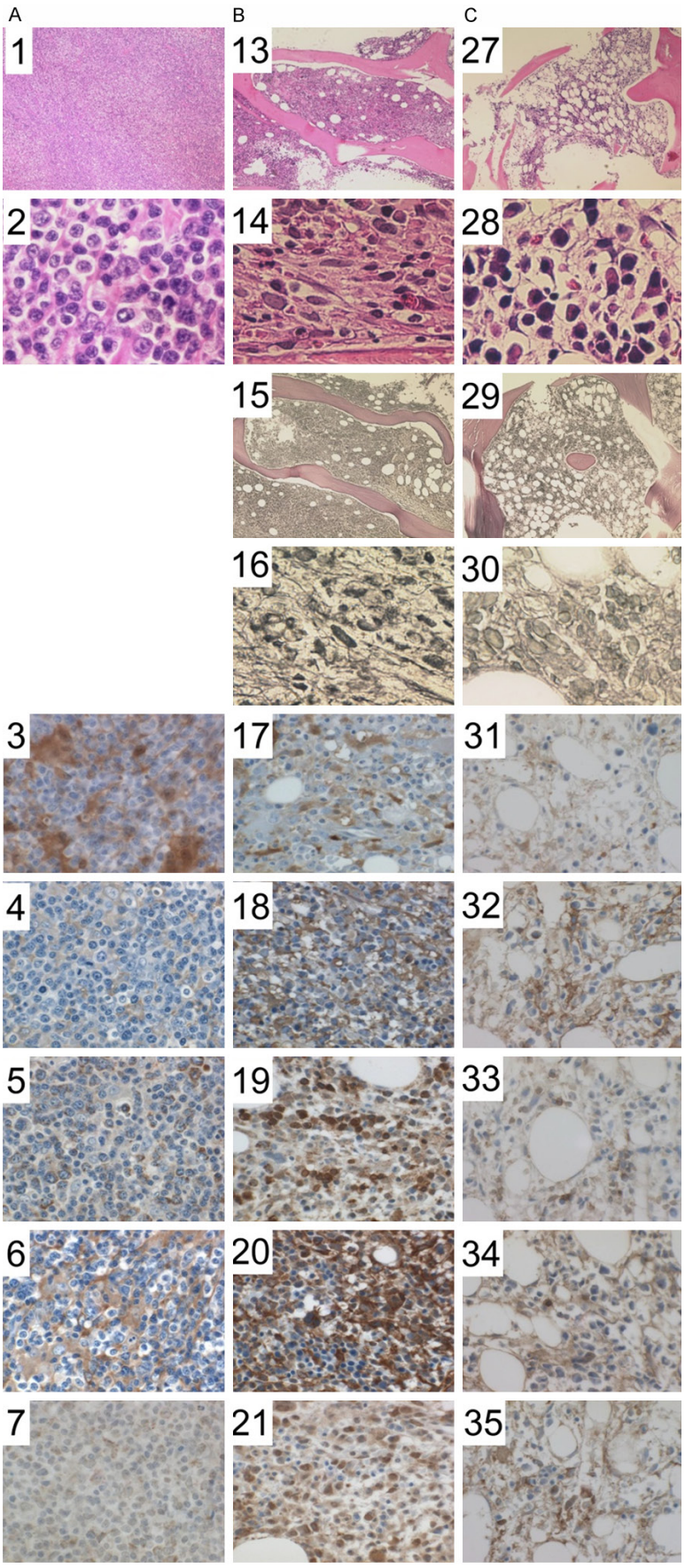


Figure 2. Histopathological findings of cervical lymph node biopsy Tin case 1. A. HE stain (×40), B. HE stain (×600): the basic architecture of the lymph node is lost and proliferation of medium- to large-sized polymorphic atypical cells is seen in a cord-like arrangement or in an isolated scattered distribution; C. CD3 positive (×600); D. CD4 positive (×600); E. PD1 positive (×600); F. CXCL13 positive (×600); G. CD21: marked hyperplasia of follicular dendritic cells is visible around the capillaries (×600); H. CD10 negative (×600); I. Ki-67 positive in a high percentage of cells (×600).

Case 2: This case was reported by us in 2013 [2]. A 65-year-old man presented to Juntendo University Urayasu Hospital with the chief complaints of fever and rash. His medical history and family history were non-contributory. The fever and rash developed in March 2012 (**Figure 4**). In April 2012, multiple superficial lymph node enlargements became evident. The patient was admitted to our department in May 2012. Test data in **Table 2**.

A left axillary lymph node biopsy was performed. Histopathological examination of sections stained with HE revealed loss of the basic architecture of the lymph node and the proliferation of medium- to large-sized atypical polymorphiccells either in a cord-like arrangement (**Figure 5A**, **5B**) or in an isolated scattered distribution. Immunohistochemical staining of the tumor cells were positive for CD3, CD4, PD1, and CXCL13 (**Figure 5C-F**), and the chromatic response of CD21 suggested marked hyperplasia of follicular dendritic cells around the capillaries (**Figure 5G**). Staining for CD10 was negative (**Figure 5H**). Positive staining for Ki-67 was observed in a large percentage of cells (**Figure 5I**). On the basis of these findings, the patient was diagnosed as having AITL. Cerebrospinal fluid examination revealed no signs of central nervous system infiltration. Because no sample could be obtained by aspiration, a bone marrow biopsy was performed. Examination of HE-stained specimens revealed AITL infiltration of the marrow (**Figure 6B13**, **6B14**). Silver impregnation staining revealed intense fibrosis (**Figure 6B15**, **6B16**). The marrow blood tested negative for the JAK-2 V617F mutation (data not shown). The clinical



stage of the disease was judged as IVB, and the PIT was group 4 (four factors were present: age, performance status [PS] 3, increased lactate dehydrogenase [LDH] levels, and marrow infiltration).

In mid-May 2012, the patient was started on treatment with prednisolone (60 mg/day), and this resulted in resolution of the fever, rash, and lymphadenopathy. Thereafter, CHOP therapy was initiated. A marrow biopsy examination done at the end of the second course of CHOP therapy (early June 2012) revealed disappearance of AITL infiltration (**Figure 6C27**, **6C28**) and alleviation of MF (**Figure 6C29**, **6C30**). Upfront auto-PBSCT was performed in August. In early September 2012, 12 days after the transplantation, the patient developed acute consciousness disturbance (Glasgow Coma Scale score 5), and magnetic resonance imaging (MRI) of the head revealed multiple tumorous shadows in the brain parenchyma. Cerebrospinal fluid examination at this time revealed AITL cells, confirming infiltration of the central nervous system by AITL. At the sametime, a systemic CT scan revealed no signs of relapse at any site other than the brain parenchyma, and a bone marrow examination revealed no signs of lymphoma infiltration (**Figure 6D41**, **6D42**) or fibrosis (**Figure 6D43**, **6D44**). After steroid pulse therapy and whole brain irradiation (45 Gy in total), the consciousness disturbance resolved and the patient was once again able to walk normally. A head MRI also

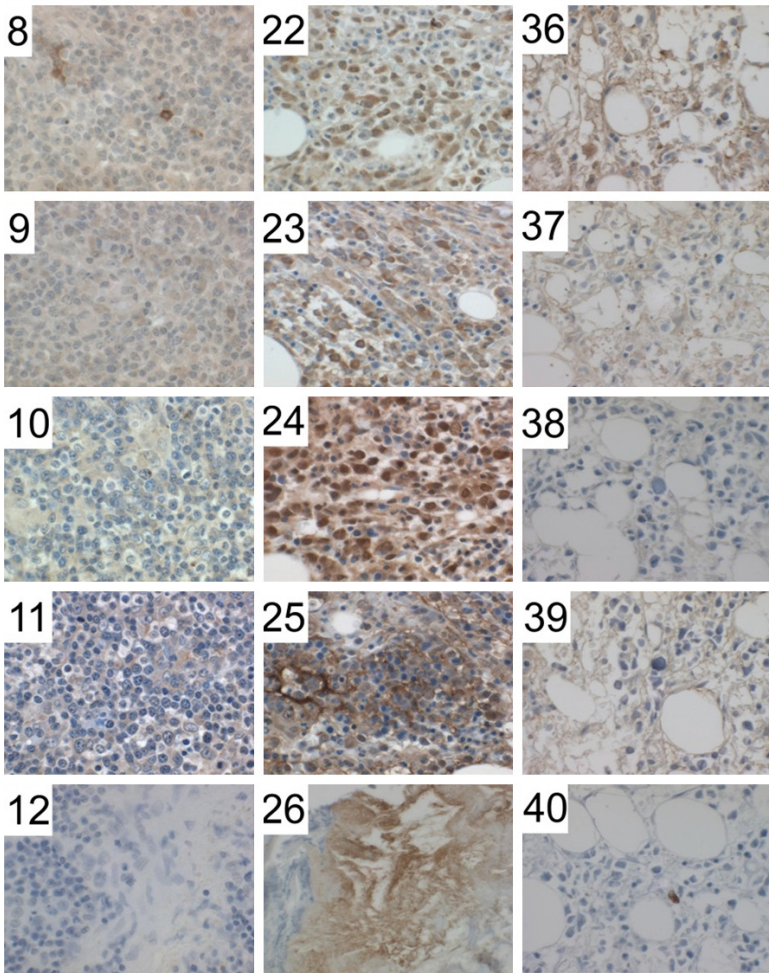


Figure 3. Histopathological findings and immunohistochemical staining for cytokines in case 1. A1-12: Lymph node biopsy before treatment, B13-26: Bone marrow biopsy before treatment, C27-40: Bone marrow biopsy on relapse. A1. HE stain (×40), A2. HE stain (×600): the basic architecture of the lymph node is lost and proliferation of medium- to large-sized atypical polymorphic cells are seen in a cord-like arrangement or in an isolated scattered distribution. A3. PDGF positive (×400); A4. b-FGF weakly positive (×400); A5. VEGF weakly positive (×400); A6. TNF-α positive (×400); A7. IFN-γ weakly positive (×400); A8. IL-1β weakly positive (×400); A9. IL-2 weakly positive (×400); A10. TGF-β weakly positive (×400); A11. IL-6 weakly positive (×400); A12. FN negative (×400); B13. HE stain (×40), B14. HE stain (×600): AITL infiltration visible; B15. Silver impregnation stain (×40), B16. Silver impregnation stain (×600): MF visible; B17. PDGF positive (×400); B18. b-FGF positive (×400); B19. VEGF positive (×400); B20. TNF-α positive (×400); B21. IFN-γ positive (×400); B22. IL-1β positive (×400); B23. IL-2 positive (×400); B24. TGF-β positive (×400); B25. IL-6 positive (×400); B26. FN weakly positive (×400); C27. HE stain (×40), C28. HE stain (×600): no evidence of AITL infiltration; C29. Silver impregnation stain (×40), C30. Silver impregnation stain (×600): no evidence of MF; C31. PDGF weakly positive (×400); C32. b-FGF weakly positive (×400); C33. VEGF weakly positive (×400); C34. TNF-α weakly positive (×400); C35. IFN-γ weakly positive (×400); C36. IL-1β weakly positive (×400); C37. IL-2 weakly positive (×400); C38. TGF-β weakly positive (×400); C39. IL-6 weakly positive (×400); C40. FN negative (×400).

revealed improvement, and the patient was discharged in October 2012. However, in May 2013, relapse of the central nervous system infiltration was detected, and the patient died.

Case 3: This case was reported by us in 2015 [16]. A 68-year-old man presented at Juntendo University Urayasu Hospital with the chief complaints of pancytopenia, erythroderma, and multiple superficial/deep lymph node enlargements. His medical and family histories contained nothing noteworthy. The patient developed generalized edema, erythroderma, and pancytopenia in June 2014 (**Figure 7**). He visited the Department of Dermatology of our hospital in July 2014. At that time, a skin biopsy led to the diagnosis of psoriatic erythroderma. Treatment with prednisolone (15 mg/day) was started, and this resulted in alleviation of the systemic edema and erythroderma. However, the pancytopenia worsened, and multiple superficial/deep lymph node enlargements also became evident. The patient was therefore admitted to our department in August 2014. Test data in **Table 3**. A left axillary lymph node biopsy was carried out, and histopathological examination of sections stained with HE revealed disappearance of the basic architecture of the lymph node (**Figure 8A**) and proliferation of medium-sized atypical cells with irregular nuclei (**Figure 8B**). Immunohistochemical staining of the tumor cells were positive for CD2, CD3, and C-C chemokine receptor type 4 (CCR4) (**Figure 8C**, **8D**, **8P**). The staining results were negative for CD4, CD5, CD7, CD8, CD10, CD20, CD56, Epstein-Barr virus-encod-

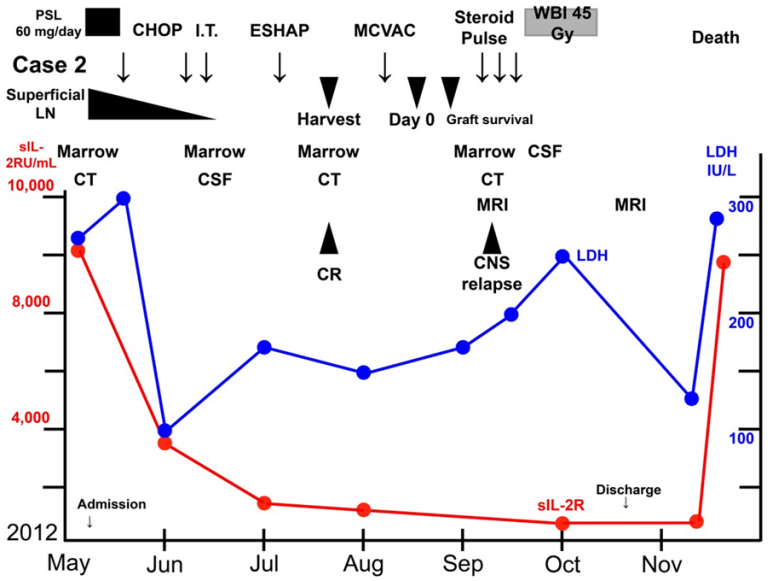


Figure 4. Clinical course of case 2. IT, intrathecal; WBI, whole brain irradiation; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; CNS, central nervous system; ESHAP, etoposide, cisplatin, cytarabine, methylprednisolone; WBI, whole brain irradiation.

ed small RNA (EBER), Granzyme B, and PD1 (**Figure 8E-J**, **8L-O**). The chromatic response of CD21 did not suggest hyperplasia of the follicular dendritic cells (**Figure 8K**). On the basis of these findings, the patient was diagnosed as having peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS). Cerebrospinal fluid examination did not reveal evidence of central nervous system infiltration. Only a very small amount of bone marrow could be collected by puncture and aspiration. Therefore, a bone marrow biopsy was performed. Analysis of the sample revealed infiltration by PTCL-NOS (**Figure 9B13**, **9B14**). Silver impregnation staining revealed MF (**Figure 9B15**, **9B16**). The marrow blood tested negative for the JAK-2 V617F mutation (data not shown). The clinical disease stage was IVB, and the PIT was group 4 (four factors were present: age, PS3, increased LDH levels, and marrow infiltration).

In early September 2014, the patient was started on the first course of CHOP therapy. Bone marrow examination was conducted and revealed a marked decrease of the PTCL-NOS infiltration (**Figure 9C27**, **9C28**) and alleviation of MF (**Figure 9C29**, **9C30**). In early October 2014, the patient was started on the second course of CHOP therapy. At this time, a bone marrow examination revealed aggravation of the PTCL-NOS infiltration (**Figure 9D41**, **9D42**) and MF (**Figure 9D43**, **9D44**). The patient was thus judged to be refractory to CHOP therapy, and treatment was switched to mogamulizumab therapy (1 mg/kg) in mid-November. Two courses of this therapy were applied; however, further elevation of LDH and soluble interleukin-2 receptor (sIL-2R) was noted, accompanied by increased superficial lymph node enlargement. In early December 2014, the therapy was switched again from mogamulizumab to cyclophosphamide 600 mg/m^2, days 1 and 8; etoposide 70 mg/m^2, days 1-3; procarbazine 60 mg/m^2, days 1-10; and prednisolone 60 mg/m^2, days 1-10 (CEPP therapy). This resulted in temporary improvement of the patient's condition. However, the lymphoma relapsed, and the patient died in January 2015.

Results

The data acquired in the treatment of these three patients allowed the time profiles of cytokine production in the PTCL cells to be analyzed.

Case 1: Immunohistochemical staining of the lymph node for cytokines was performed before CHOP therapy and waspositive for PDGF and TNF-α (**Figure 3A3**, **3A6**; **Table 4**). Before the CHOP therapy was started, the bone marrow showed AITL infiltration (**Figure 3B13**, **3B14**) and MF (**Figure 3B15**, **3B16**) was observed. Strongly positive staining was observed for all cytokines except for fibronectin (FN), which was only weakly positive (**Figure 3B17-26**). No immunohistochemical staining of the bone marrow was performed at the time of remission. On-relapse, the bone marrow showed no infiltration (**Figure 3C27**, **3C28**) and complete resolution of MF (**Figure 3C29**, **3C30**). Immunostaining was negative for FN only (**Figure 3C40**) and weakly positive for all other cytokines (**Figure 3C31-39**). The results are summarized in **Table 4**.

Table 2. Test data for case 2

Blood count			
WBC	3400/μL↓	Hb	10.5 g/dL↓
Band	9.0%	Ht	31.8%↓
Seg	68.0%↓	MCV	85.1%
Lym	13.0%↓	MCH	28.2%
Mono	10.0%↑	Plt	10.0×10^4/μL↓
RBC	374×10^4/μL↓	Retic	0.1%↓
Biochemistry			
TP	6.3 g/dL↓	γ-GTP	46 IU/L
Alb	2.5 g/dL↓	T-Bil	0.4 mg/dL
AST	17 IU/L	BUN	14 mg/dL
ALT	24 IU/L	Cr	0.82 mg/dL
LDH	259 IU/L↓	CRP	1.0 mg/dL↑
ALP	161 IU/L	Ferritin	544.2 ng/mL↑
Immunoserological findings			
IgG	2588 mg/dL↑	sIL-2R	9140 U/mL↑
IgA	297 mg/dL	Direct Coomb's	negative
IgM	196 mg/dL	Haptoglobin	211 mg/dL↓
Antinuclear antibody	1:40	HTLV-1 antibody	Negative
PAIgG	83 ng/10^7 cell↑	HIV antibody	Negative
Coagulation profile			
PT	69%↓	APTT	31.1 s
Cytokines			
PDGF-AB	11,400 pg/mL (10, 499-29, 463)	High-sensitivity TNF-α	1.9 pg/mL (0.550-2.816)
VEGF	89 pg/mL (ND-115)	IL-6	4.2↑ pg/mL (≤4.0)
TGF-β1	11.7↓ ng/mL (903-1654)		

↑, upper limit; ↓, lower limit. No abnormality was found on urinalysis. Lymph node and whole blood chromosomes (G-band): 46, XY. On Southern blotting of lymph nodes and whole blood, gene rearrangement noted for TCRCβ1, but not noted for IG (H) JH. Band, banding; Seg, segment; RBC, red blood cells; Ht, hematocrit; Retic, reticulocyte; PAIgG, platelet associated immunoglobulin G; PT, prothrombin time; APTT, activated partial thromboplastin time; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; TGF, transforming growth factor; TNF-α, tumor necrosis factor-α; IL, interleukin; TCRC β1, T-cell receptor Cβ1; IG(H)JH, immunoglobulin heavy chain.

Case 2: Immunohistochemical staining of the lymph node for cytokines was performed before CHOP therapy and was positive for PDGF, interleukin (IL)-2, and FN (**Figure 6A3**, **6A9**, **6A12**; **Table 5**). Weakly positive staining was noted for b-FGF and IL-6 (**Figure 6A4**, **6A11**). Staining was negative for VEGF, TNF-α, interferon (IFN)-g, IL-1β, and TGF-β (**Figure 6A5-8**, **6A10**). Before the CHOP therapy, bone marrow examination revealed AITL infiltration (**Figure 6B13**, **6B14**) and MF (**Figure 6B15**, **6B16**). Staining was positive for PDGF, b-FGF, and IL-2 (**Figure 6B17**, **6B18**, **6B23**). Staining for VEGF and FN was weakly positive (**Figure 6B19**, **6B26**), and staining for TNF-α, IFN-γ, IL-1β, TGF-β, and IL-6 was negative (**Figure 6B20-22**, **6B24**, **6B25**). At the time of remission, the bone marrow no longer showed either infiltration (**Figure 6C27**, **6C28**) or MF (**Figure 6C29**, **6C30**). Strongly positive staining was observed for b-FGF, IL-2, and FN (**Figure 6C32**, **6C37**, **6C40**); weakly positive staining for IL-1β (**Figure 6C36**); and negative staining for PDGF, VEGF, TNF-α, IFN-γ, TGF-β, and IL-6 (**Figure 6C31**, **6C33-35**, **6C38**, **6C39**). Even after relapse, the bone marrow showed no evidence of infiltration (**Figure 6D41**, **6D42**) or MF (**Figure 6D43**, **6D44**). Strongly positive staining was noted for b-FGF, IL-2, and FN (**Figure 6D46**, **6D51**, **6D54**); weakly positive staining for IL-1β, TGF-β, and

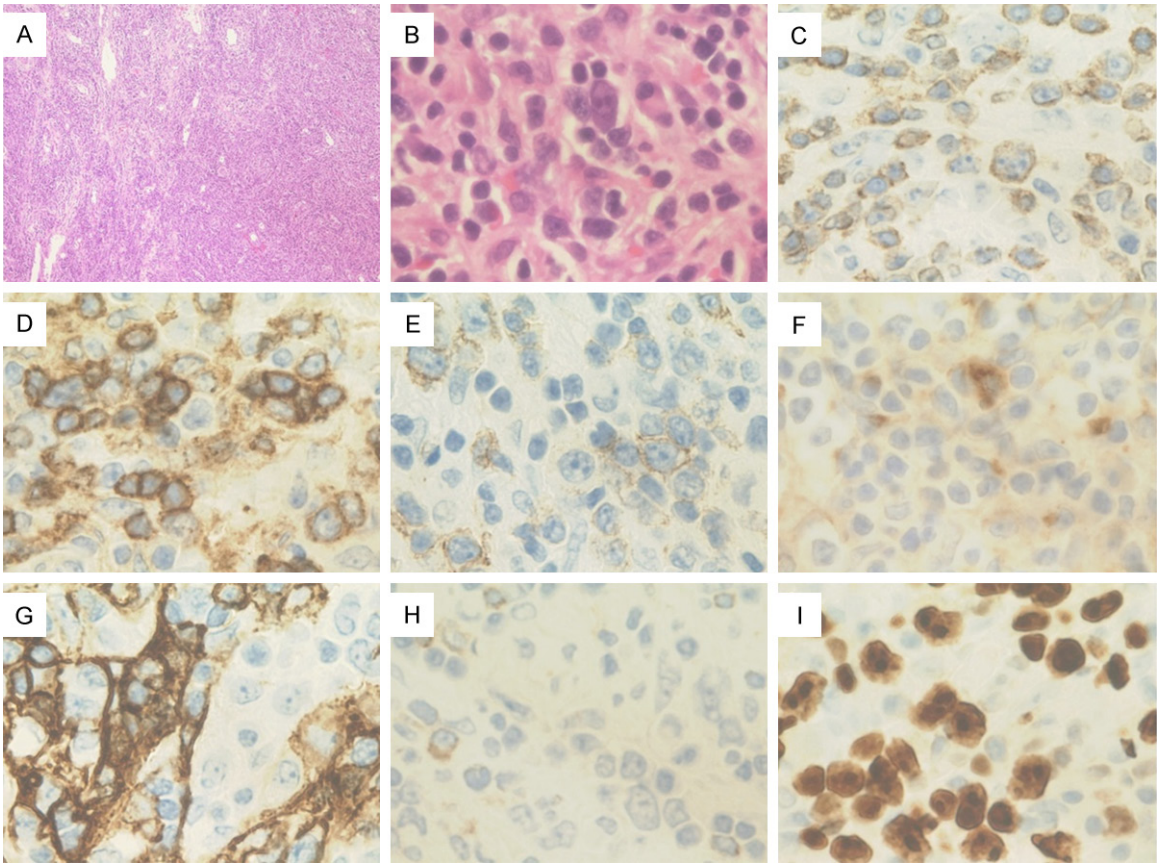
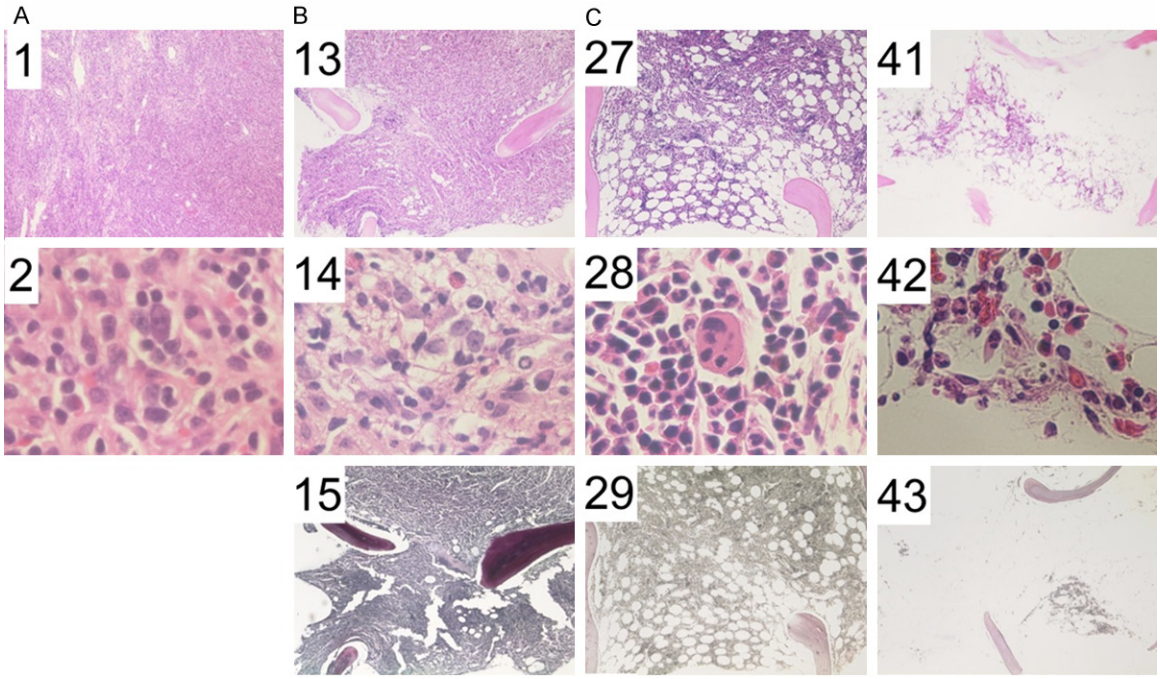
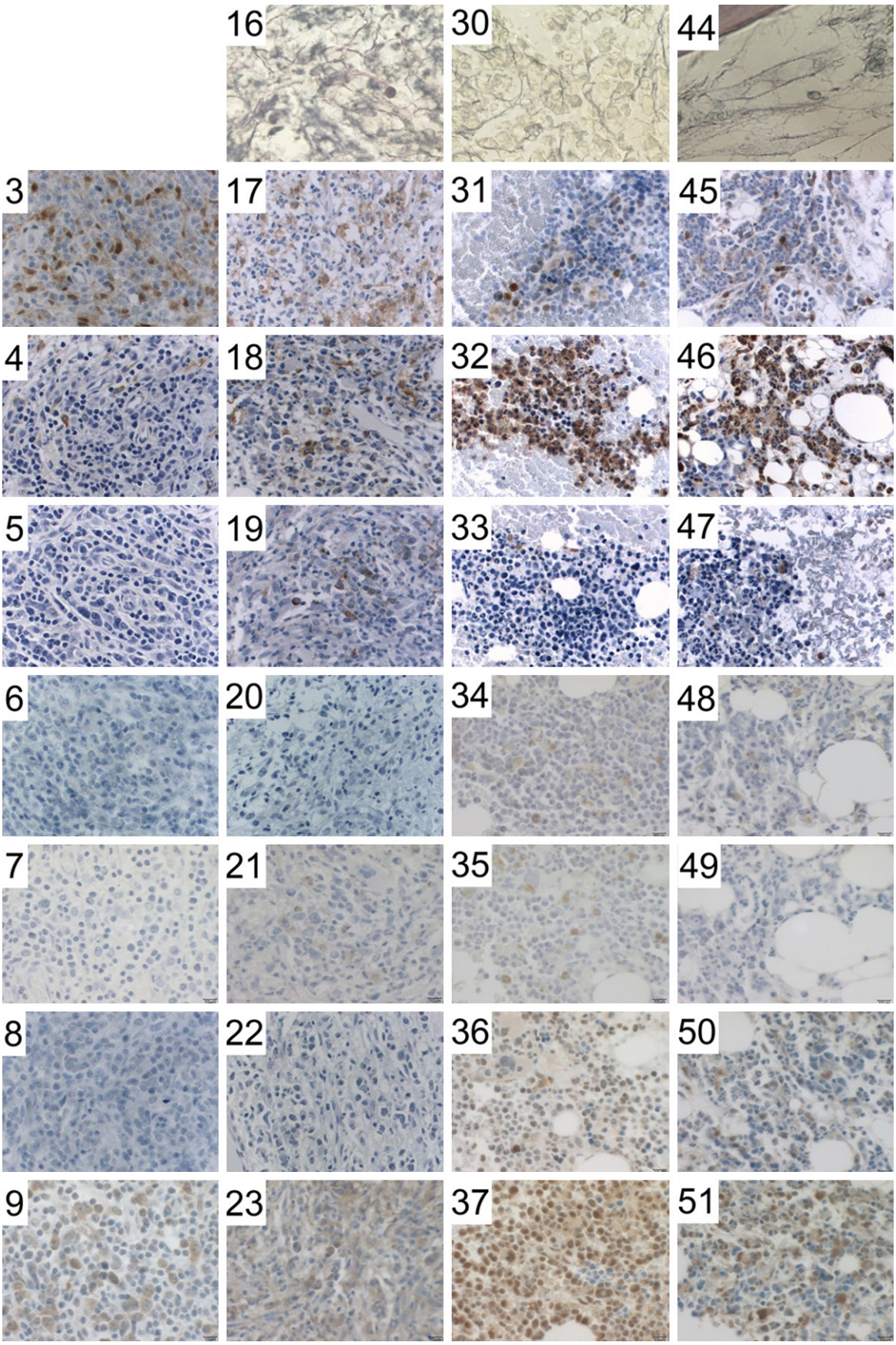


Figure 5. Histopathological findings of axillary lymph node biopsy in case 2. A. HE stain (×40), B. HE stain (×600): the basic architecture of the lymph node is lost and proliferation of medium- to large-sized atypical polymorphiccell-sis seen in a cord-like arrangement or in an isolated scattered distribution; C. CD3 positive (×600); D. CD4 positive (×600); E. PD1 positive (×600); F. CXCL13 positive (×600); G. CD21 marked hyperplasia of the follicular dendritic cells visible around the capillaries (×600); H. CD10 negative (×600); I. Ki-67 positive in a high percentage of cells (×600).

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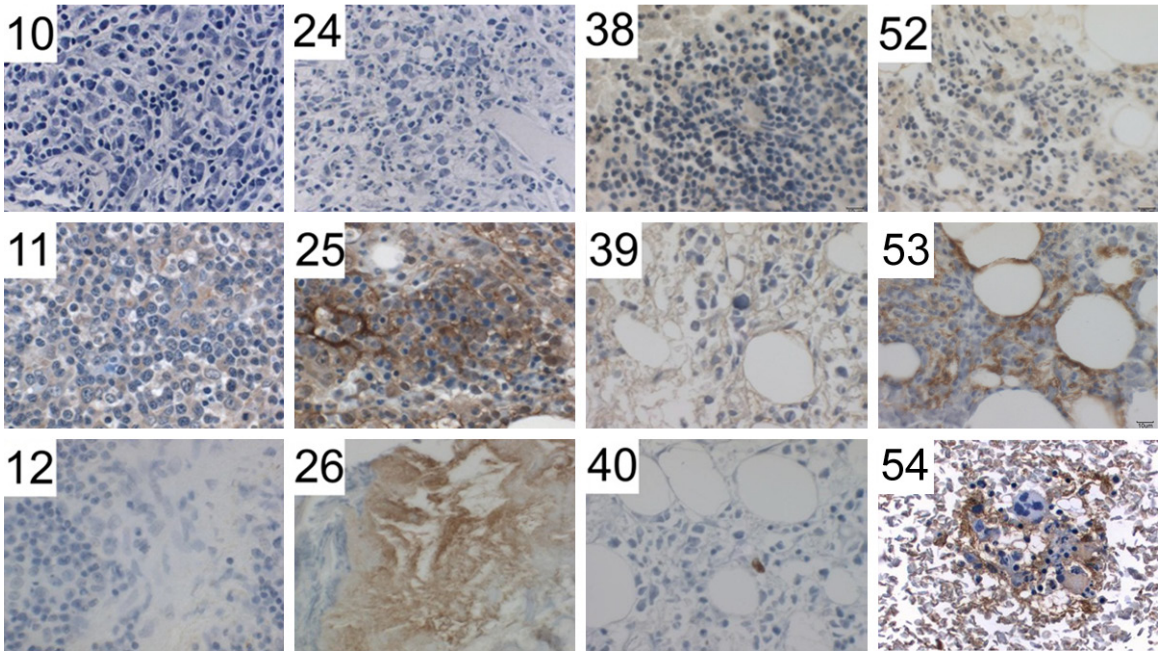


Figure 6. Histopathological findings and immunohistochemical staining for cytokines in case 2. A1-12: Axillary lymph node biopsy, B13-26: Bone marrow biopsy before treatment, C27-40: Bone marrow biopsy on complete remission, D41-54: Bone marrow biopsy on relapse. A1. HE stain (×40), A2. HE stain (×600): The basic architecture of the lymph node is lost and proliferation of medium- to large-sized atypical polymorphiccellsis seen in a cord-like arrangement or in an isolated scattered distribution; A3. PDGF positive (×400); A4. b-FGF weakly positive (×400); A5. VEGF negative (×400); A6. TNF-α negative (×400); A7. IFN-γ negative (×400); A8. IL-1β negative (×400); A9. IL-2 positive (×400); A10. TGF-β negative (×400); A11. IL-6 weakly positive (×400); A12. FN positive (×400); B13. HE stain (×40), B14. HE stain (×600): AITL infiltration visible; B15. Silver impregnation stain (×40), B16. Silver impregnation stain (×600): MF visible; B17. PDGF positive (×400); B18. b-FGF positive (×400); B19. VEGF weakly positive (×400); B20. TNF-α negative (×400); B21. IFN-γ negative (×400); B22. IL-1β negative (×400); B23. IL-2 positive (×400); B24. TGF-β negative (×400); B25. IL-6 negative (×400); B26. FN weakly positive (×400); C27. HE stain (×40), C28. HE stain (×600): no evidence of AITL infiltration; C29. Silver impregnation stain (×40), C30. Silver impregnation stain (×600): no evidence of MF; C31. PDGF negative (×400); C32. b-FGF positive (×400); C33. VEGF negative (×400); C34. TNF-α negative (×400); C35. IFN-γ negative (×400); C36. IL-1β weakly positive (×400); C37. IL-2 positive (×400); C38. TGF-β negative (×400); C39. IL-6 negative (×400); C40. FN positive (×400); D41. HE stain (×40), D42. HE stain (×600): AITL infiltration absent; D43. Silver impregnation stain (×40), D44. Silver impregnation stain (×600): MF absent; D45. PDGF negative (×400); D46. b-FGF positive (×400); D47. VEGF negative (×400); D48. TNF-α negative (×400); D49. IFN-γ negative (×400); D50. IL-1β weakly positive (×400); D51. IL-2 positive (×400); D52. TGF-β weakly positive (×400); D53. IL-6 weakly positive (×400); D54. FN positive (×400).

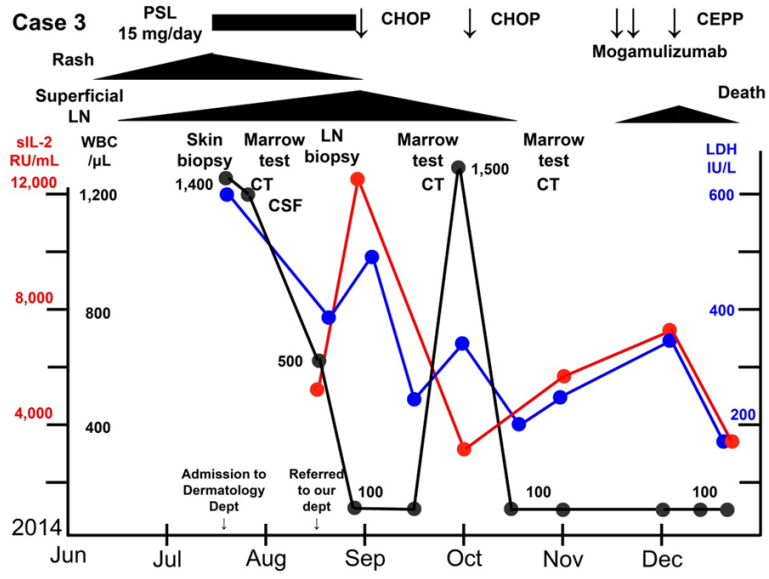


Figure 7. Clinical course of case 3. CEPP, cyclophosphamide, etoposide, procarbazine, prednisolone. LN, lymph node.

IL-6 (**Figure 6D50**, **6D52**, **6D53**); and negative staining for PDGF, VEGF, TNF-α, and IFN-γ (**Figure 6D45**, **6D47-49**). The results are summarized in **Table 5**.

Case 3: Immunohistochemical staining of the lymph node for cytokines was performed before CHOP therapy and was positive for PDGF, b-FGF, VEGF, TNF-α, IFN-γ, IL-1β, IL-2,

Table 3. Test data for case 1

Blood count			
WBC	500/μL↓	RBC	311×10⁴/μL↓
Band	16.0%↑	Hb	10.3 g/dL↓
Seg	53.0%	Ht	32.0%↓
Lym	27.0%	MCV	102.9 fl↑
Mono	1.0%↓	MCH	33.1 pg
Eos	2.0%	Plt	6.8×10⁴/μL↓
Bas	1.0%	Retic	1.1%
Biochemistry			
TP	5.1 g/dL↓	γ-GTP	22 IU/L
Alb	3.0 g/dL↓	T-Bil	0.6 mg/dL
AST	24 IU/L	BUN	16 mg/dL
ALT	31 IU/L	Cr	0.90 mg/dL
LDH	398 IU/L↑	CRP	1.9 mg/dL↑
ALP	169 IU/L	Ferritin	372.0 ng/mL
Serum cytokines			
PDGF-AB	845 pg/mL↓ (10, 499-29, 463)	b-FGF	≤10 pg/mL (≤10)
VEGF	33 pg/mL↓ (62-707)	IL-6	5.7 pg/mL↑ (≤4.0)
TGF-β1	2.40 ng/mL (1.56-3.24)	IL-10	125 pg/mL↑ (ND-5)
High-sensitivity TNF-α	12.3 pg/mL↑ (0.550-2.816)		
Coagulation profile			
PT	80%	FDP	8.8 μg/mL↑
APTT	32.8 s	DD	3.64 μg/mL↑
Fbg	203 mg/dL	AT3	53%↓
Immunoserological findings			
IgG	851 mg/dL↓	sIL-2R	5410 U/mL↑
IgA	156 mg/dL	HTLV-1 antibody	Negative
IgM	25 mg/dL↓	HIV antibody	Negative
Antinuclear antibody	1:40		

↑, upper limit ; ↓, lower limit; No abnormality was found on urinalysis. Lymph nodes analysis: G-band - 46, Y, add (X) (q22), del (6) (q?), -9, inv (9) (p12q13), del (11) (q?), -12, add (13) (q22), add (16) (q12.1), add (18) (q21), +mar1, +mar2; TCRCβ1 - gene rearrangement; IG (H) JH - no gene rearrangement; CCR4 immunostaining - positive. Bone marrow analysis: G-Band - poor proliferation; TCRC β1 and IG (H) JH - samples insufficient for testing; JAK2V617F - no mutation (paraffin block sample). b-FGF, basic fibroblast growth factor; add, additional material of unknown origin; del, deletion; mar, marker chromosome; CCR4, C-C chemokine receptor type 4.

and TGF-β (**Figure 9A3-10**; **Table 6**) and negative for IL-6 and FN (**Figure 9A11**, **9A12**). Before the start of CHOP therapy, the bone marrow showed PTCL-NOS infiltration (**Figure 9B13**, **9B14**) and MF (**Figure 9B15**, **9B16**). Strongly positive staining for PDGF, b-FGF, VEGF, TNF-α, IFN-γ, IL-1β, IL-2, and TGF-β was noted (**Figure 9B17-24**), whereas staining was negative for IL-6 and FN (**Figure 9B25**, **9B26**). During the period of partial response (PR) after the first course of CHOP therapy, both infiltration (**Figure 9C27**, **9C28**) and MF (**Figure 9C29**, **9C30**) were no longer seen in the bone marrow. Positive staining was observed for b-FGF, IFN-γ, IL-1β, IL-2, and IL-6 (**Figure 9C32**, **9C35-37**, **9C39**), whereas staining was negative for PDGF, VEGF, TNF-α, TGF-β, and FN (**Figure 9C31**, **9C33**, **9C34**, **9C38**, **9C40**). When relapse occurred after the second course of CHOP therapy, relapse of infiltration (**Figure 9D41**, **9D42**) and MF (**Figure 9D43**, **9D44**) was noted in the bone marrow, with positive staining for PDGF, b-FGF, TNF-α, IFN-γ, IL-1β, TGF-β, and IL-6 (**Figure 9D45**, **9D46**, **9D48-50**, **9D52**, **9D53**), and negative staining for VEGF, IL-2, and FN (**Figure 9D47**, **9D51**, **9D54**). The results are summarized in **Table 6**.

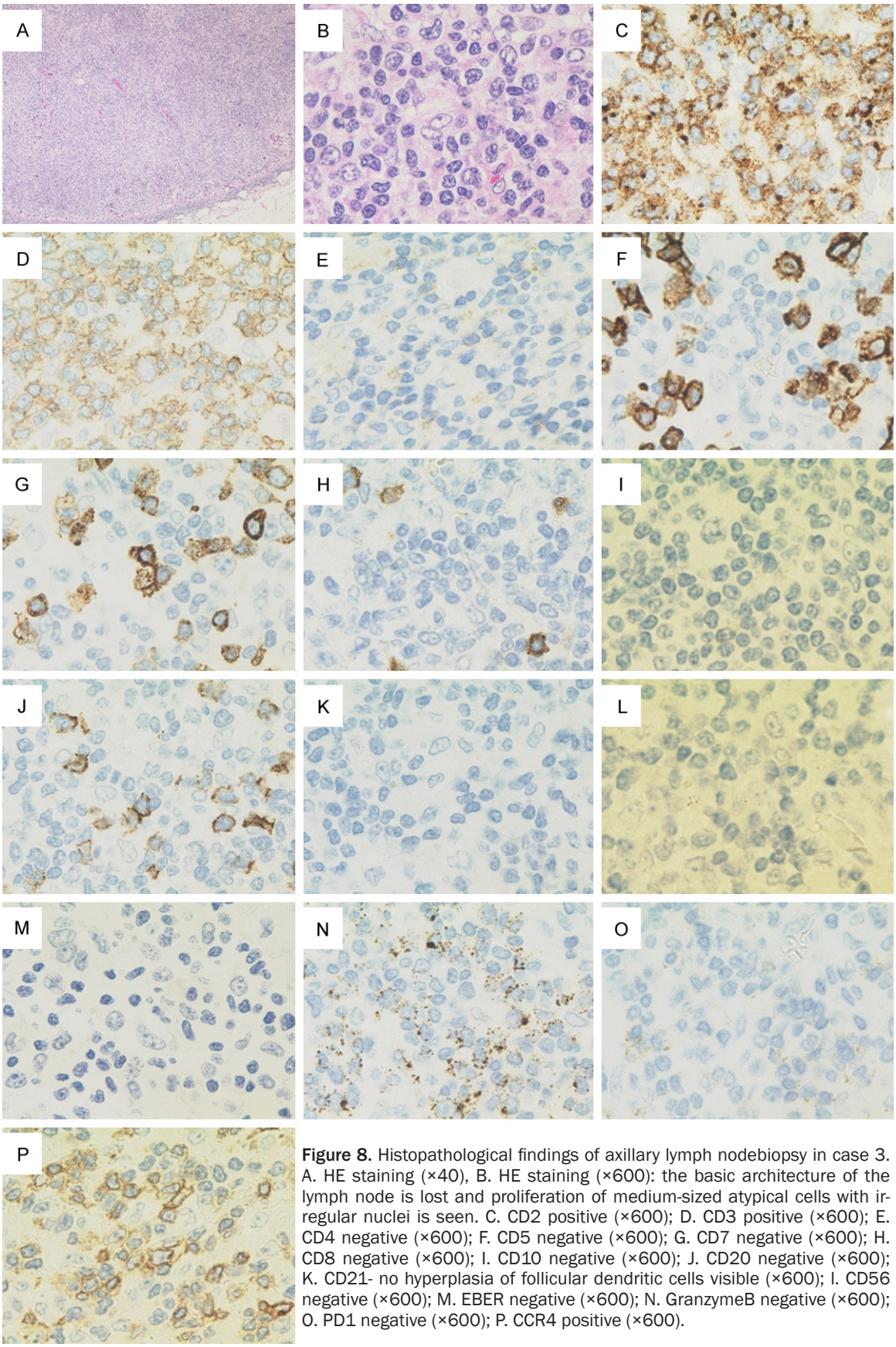
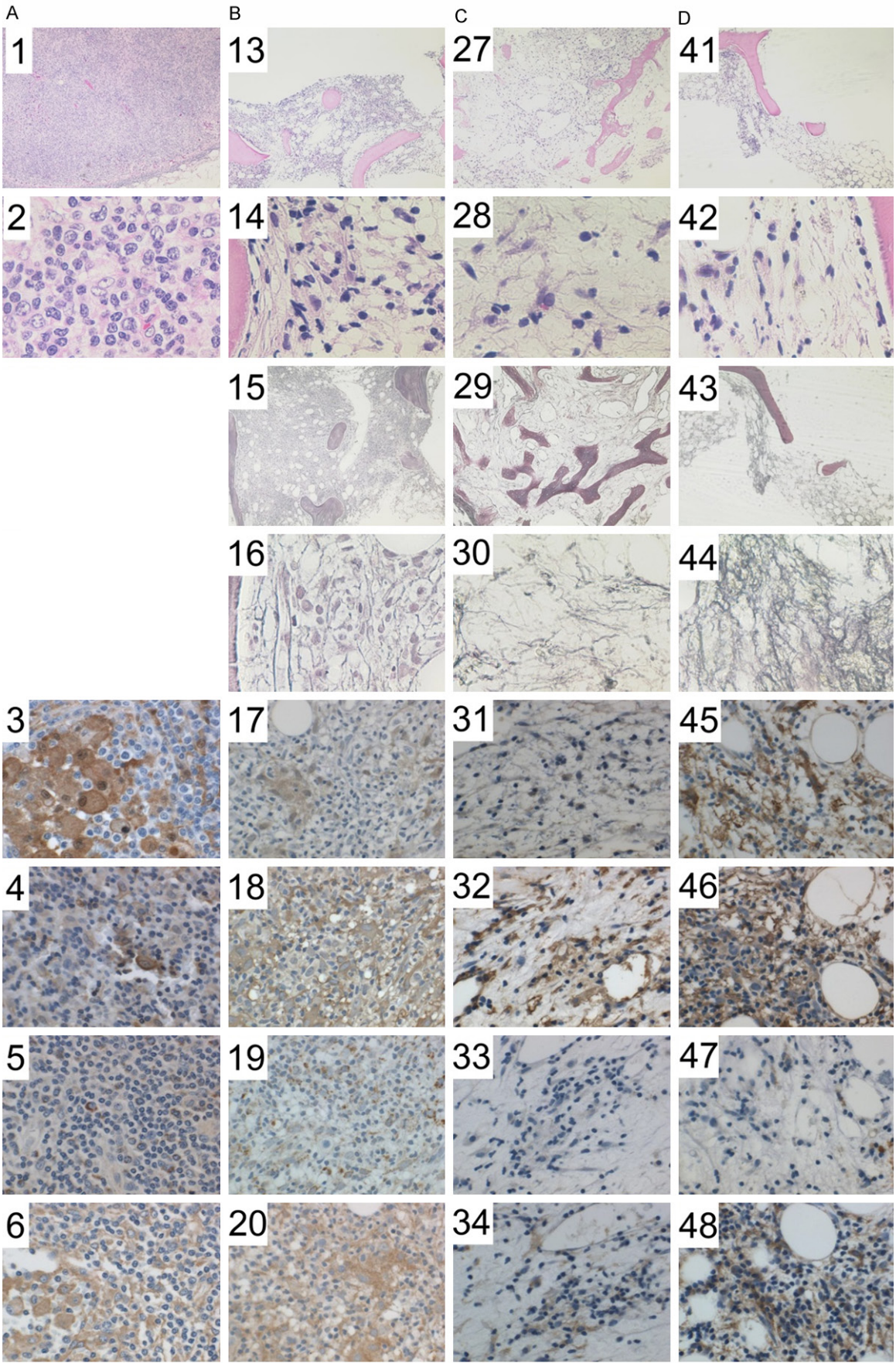


Figure 8. Histopathological findings of axillary lymph nodebiopsy in case 3. A. HE staining (×40), B. HE staining (×600): the basic architecture of the lymph node is lost and proliferation of medium-sized atypical cells with irregular nuclei is seen. C. CD2 positive (×600); D. CD3 positive (×600); E. CD4 negative (×600); F. CD5 negative (×600); G. CD7 negative (×600); H. CD8 negative (×600); I. CD10 negative (×600); J. CD20 negative (×600); K. CD21- no hyperplasia of follicular dendritic cells visible (×600); l. CD56 negative (×600); M. EBER negative (×600); N. GranzymeB negative (×600); O. PD1 negative (×600); P. CCR4 positive (×600).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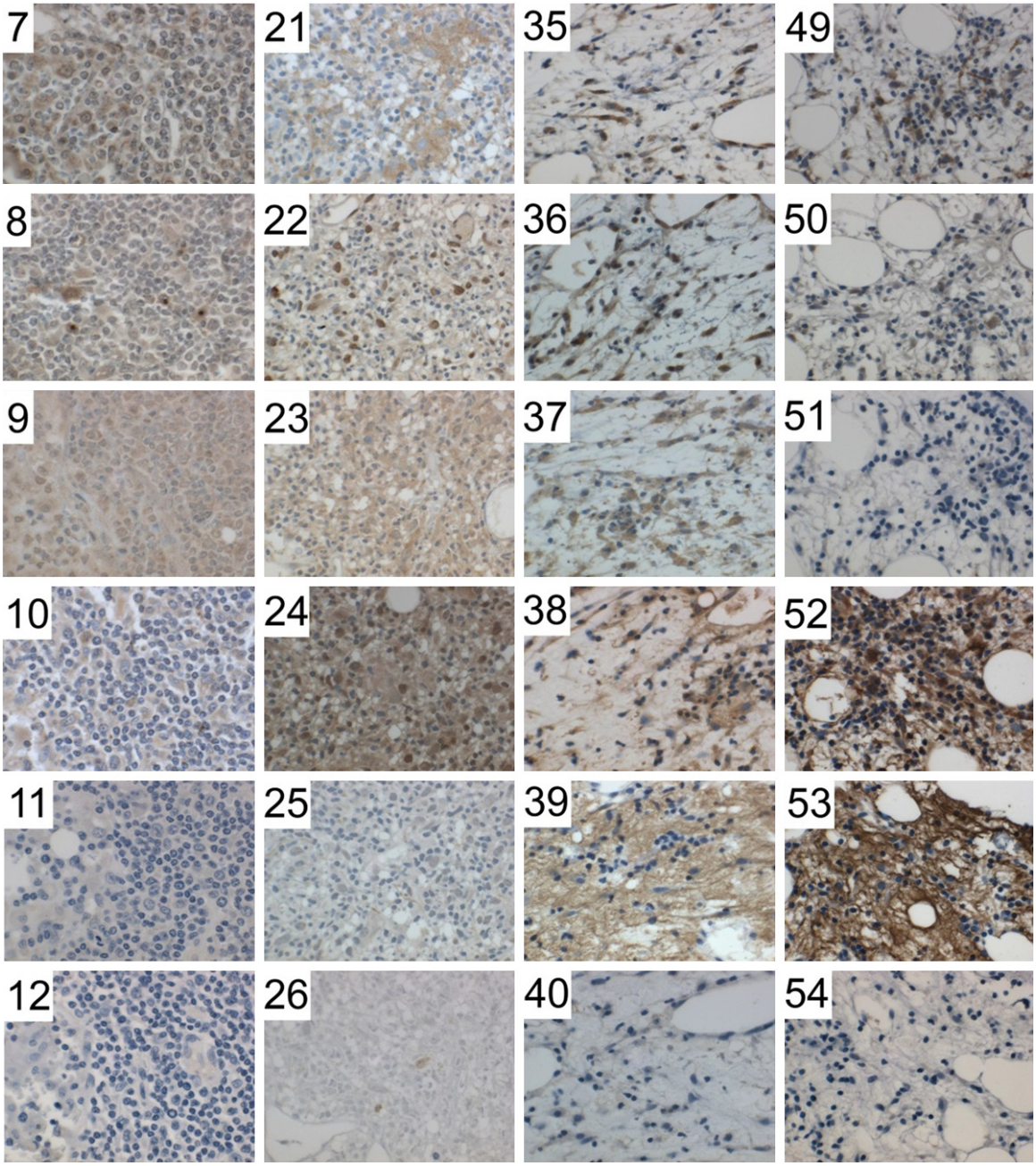

Figure 9. Histopathological findings and immunohistochemical staining for cytokines in case 3. A1-12: Axillary lymph node biopsy, B13-26: Bone marrow biopsy before treatment, C27-40: Bone marrow biopsy on remission, D41-54: Bone marrow biopsy on relapse. A1. HE stain (×40), A2. HE stain (×600): the basic architecture of the lymph node is lostandproliferation of medium-sized atypical cells with irregular nuclei is seen. A3. PDGF positive (×400); A4. b-FGF positive (×400); A5. VEGF positive (×400); A6. TNF-α positive (×400); A7. IFN-γ positive (×400); A8. IL-1β positive (×400); A9. IL-2 positive (×400); A10. TGF-β positive (×400); A11. IL-6 negative (×400); A12. FN negative (×400); B13. HE stain (×40), B14. HE stain (×600): PTCL-NOS infiltration visible; B15. Silver impregnation stain (×40), B16. Silver impregnation stain (×600): evidence of MF visible; B17. PDGF positive (×400); B18. b-FGF positive (×400); B19. VEGF positive (×400); B20. TNF-α positive (×400); B21. IFN-γ positive (×400); B22. IL-1β positive (×400); B23. IL-2 positive (×400); B24. TGF-β positive (×400); B25. IL-6 negative (×400); B26. FN negative (×400); C27. HE stain (×40), C28. HE stain (×600): marked decrease of PTCL-NOS infiltration visible; C29. Silver impregnation stain (×40), C30. Silver impregnation stain (×600): evidence of improvement of the MF; C31. PDGF negative (×400); C32. b-FGF positive (×400); C33. VEGF negative (×400); C34. TNF-α negative (×400); C35. IFN-γ positive (×400); C36. IL-1β positive (×400); C37. IL-2 positive (×400); C38. TGF-β negative (×400); C39. IL-6 positive (×400); C40. FN negative (×400); D41. HE stain (×40), D42. HE stain (×600): evidence of aggravation of the PTCL-NOS infiltration; D43. Silver

impregnation stain (×40), D44. Silver impregnation stain (×600); evidence of aggravation of the MF. D45. PDGF positive (×400); D46. b-FGF positive (×400); D47. VEGF negative (×400); D48. TNF-α positive (×400); D49. IFN-γ positive (×400); D50. IL-1β positive (×400); D51. IL-2 negative (×400); D52. TGF-β positive (×400); D53. IL-6 positive (×400); D54. FN negative (×400).

Table 4. Summary of the findings of immunohistochemical staining of the lymph nodes and bone marrow for cytokines in case 1

Case 1	LN	BM	BM	BM
	Before CHOP	Before CHOP	On remission	On relapse
Marrow infiltration	ND	+	ND	-
BMF	ND	+	ND	-
PDGF	+	+	ND	±
b-FGF	±	+	ND	±
VEGF	±	+	ND	±
TNF-α	+	+	ND	±
IFN-γ	±	+	ND	±
IL-1β	±	+	ND	±
IL-2	±	+	ND	±
TGF-β	±	+	ND	±
IL-6	±	+	ND	±
FN	-	±	ND	-

+, positive; ±, pseudo positive; -, negative; LN, lymph node; BM, bone marrow; CHOP, cyclophosphamide, adriamycin, vincristine, and prednisolone therapy; BMF, bone marrow fibrosis; ND, not done; IFN, interferon; FN, fibronectin.

Table 5. Summary of the findings of immunohistochemical staining of the lymph nodes and bone marrow for cytokines in case 2

Case 2	LN	BM	BM	BM
	Before CHOP	Before CHOP	On remission	On relapse
Marrow infiltration		+	-	-
BMF		+	-	-
PDGF	+	+	-	-
b-FGF	±	+	+	+
VEGF	-	±	-	-
TNF-α	-	-	-	-
IFN-γ	-	-	-	-
IL-1β	-	-	±	±
IL-2	+	+	+	+
TGF-β	-	-	-	±
IL-6	-	-	-	±
FN	+	±	+	+

+, positive; ±, pseudo positive; -, negative.

Discussion

According to our literature search, 16 cases (including our three cases) of T-cell lymphoma complicated by MF have been reported to date (i.e., 15 previously reported cases and our current case 1) (**Table 7**). The most frequent histological type was AITL (eightcases), followed by PTCL (six cases). In addition, there was one case of cytotoxic T-cell lymphoma and one case of T-cell lymphoma. The disease was stage III in 2 cases and stage IV in 13 cases, indicating that in all cases except one, the disease was at an advanced stage. Bone marrow infiltration was seen frequently (present in 13 cases and absent in 3 cases). All three of the cases encountered at our department had bone marrow infiltration. Examination of cytokines, which are considered to be the cause of MF, was done in only seven cases. Examination of cytokines was performed in the serum only in three cases (cases 6, 9, and 16), immunostaining only was performed in three cases (cases 2, 3, and 5), and serum examination and immunostaining was performed in one case (case 1). No examination for cytokines was performed in nine cases (cases 4, 7, 8, and 10-15). Staining for PDGF was positive in fivecases, TGF-β was positive in four cases, TNF-α was positive in two cases, and b-FGF was positive in one case, although some cases showed positive staining for two or more of these cytokines. The possible mechanisms for the onset of MF are (1) induction by cytokines produced by the lymphoma cells infiltrating the bone marrow, (2) induction via by cytokines produced by lymphoma cells remote from the marrow in the absence of infiltration of the bone marrow by lymphoma cells, or (3) a combination of mechanisms (1) and (2). In the future, we plan to carry out chronological examination for cytokines in the serum and carry out immunostaining for cytokines not only in cases of T-cell lymphoma but also in cases of malignant lymphoma complicated and not complicated by MF.

Table 6. Summary of findings of immunohistochemical staining of the lymph nodes and bone marrow for cytokines in case 3

Case 3	LN	BM	BM	BM
	Before CHOP	Before CHOP	On partial remission after first course of CHOP	After 2 courses of CHOP
Marrow infiltration	ND	+	-	+
BMF	ND	+	-	+
PDGF	+	+	-	+
b-FGF	+	+	+	+
VEGF	+	+	-	-
TNF-α	+	+	-	+
IFN-γ	+	+	+	+
IL-1β	+	+	+	+
IL-2	+	+	+	-
TGF-β	+	+	-	+
IL-6	-	-	+	+
FN	-	-	-	-

+, positive; ±, pseudo positive; -, negative.

Table 7. Details of the 16 reported cases of T-cell lymphoma complicated by myelofibrosis

Case	Age/ sex	Histological type	Stage	BM infiltration	Cytokine	Ref.
1	64/M	AITL	IV	+	PDGF (immunostained) TNF-α	Current article
2	65/M	AITL	IV	+	PDGF (immunostained)	[2]
3	68/M	PTCL-NOS	IV	+	PDGF (immunostained) TNF-α TGF-β	[16]
4	67/F	PTCL-NOS	IV	+	NA	[4]
5	90/M	PTCL-U	IV	+	b-FGF (immunostained)	[17]
6	68/M	PTCL-U	IV	+	TGF-β (serum)	[13]
7	69/M	PTCL	IV	+	NA	[5]
8	46/F	T-cell lymphoma (no details)	IV	+	NA	[6]
9	19/F	Cytotoxic T-cell lymphoma	IV	+	PDGF (serum) TGF-β	[14]
10	65/F	PTCL	IV	+	NA	[7]
11	63/M	AITL	IV	+	NA	[8]
12	NA	AITL	II or greater	–	NA	[9]
13	69/F	AITL	III	–	NA	[10]
14	47/M	AITL	IV	+	NA	[11]
15	55/F	AITL	IV	+	NA	[12]
16	56/M	AITL	III	–	PDGF (serum) TGF-β	[15]

Cases 1-3 in this table are cases 1-3 as described in the current article. BM, bone marrow; PTCL-NOS, peripheral T-cell lymphoma-not otherwise specified; NA, not available; PTCL-U, peripheral T-cell lymphoma-unspecified; AITL, angioimmunoblastic T-cell lymphoma.

Disclosure of conflict of interest

None.

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